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# Comparison of physicochemical characteristics between white-belly and white-core rice grains

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Using white-belly and white-core mutants of a japonica rice cultivar Wuyujing3, this study was conducted to compare the physicochemical properties of grains differing in chalkiness type. Chalky grains were larger in length, width, and thickness than the translucent grains, and consequently had higher weight. The notable differences were observed for chemical compositions, with chalky grains showing lower contents of starch and protein than the translucent. Similar trends were noted in the majority of the 17 amino acids examined and contents of manganese (Mn), potassium (K) and magnesium (Mg), suggesting the important role of storage compounds in chalkiness formation. White-belly grains differed from white-core grains in chemical components, with the former having higher amylose contents and lower Zn content. Additionally, white-core grains exhibited markedly lower contents of amino acids derived from oxaloacetate and phosphoenolpyruvate like phenylalanine, aspartate and threonine. However, no noticeable differences were detected between white-belly and translucent grains. Our results indicate different underlying mechanisms of white-belly and white-core, suggesting the necessity of comparing white-belly and white-core in the studies on chalkiness. In addition, future study should focus on interpreting the active role of protein accumulation in chalkiness formation from perspective of interactions of carbon and nitrogen metabolism.



## 1. Introduction

Chalkiness in rice grains refers to opaque part, and it is an important quality trait that determines rice price. In addition to undesirable grain appearance, chalkiness generally lowers rice milling quality, and deteriorates eating quality because of the loose endosperm structure (Singh et al., 2003; Lanning et al., 2011). Chalkiness is affected by both genotypes and growing environment, in particular the ambient temperature during critical grain-filling stages (Yamakawa and Hakata, 2010). Elevated daytime or night-time temperature during grain filling contributed to an increased incidence of chalky grains (Yamakawa and Hakata, 2010; Lanning et al., 2011). Therefore, eliminating grain chalkiness is of great importance in rice production in the scenario of projected global warming (Fitzgerald et al., 2009).

Previous studies reported the distinct differences between chalky and translucent grains in endosperm morphology, physical properties, chemical composition, and cooking and eating quality (Lisle et al., 2000; Singh et al., 2003). In the chalky endosperm, compound starch granules were loosely packed with numerous air spaces, while in the translucent tissue, starch granules were tightly packed. Chalky grains are larger in weight and bulk density, but contain less amylose, more amylopectin and short branch-chain amylopectin than translucent grains (Pantindol and Wang, 2003; Singh et al., 2003). However, Lisle et al. (2000) suggested that these differences in chemical composition did not cause significant differences in cooking and eating quality between chalky and translucent grains.

Chalky rice is generally categorized into white-belly rice, white-core rice, white-base rice, white-back rice and so on, according to the position of opaque part (Tashiro and Wardlaw, 1991). Among them, white-belly and white-core are the two leading chalky types

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in japonica rice especially in southern China. Our previous work showed that white-belly tissue differed from white-core tissue in endosperm morphology, with the latter containing fewer protein bodies whereas the former having numerous protein bodies surrounded by starch granules (Qiao et al., 2011; Xi et al., 2014). Up to now, little information is available on the differences in chemical composition between white-belly and white-core grains, although it is imperative for the elucidation of mechanism of chalkiness formation. Using mutants with high occurrences of white-belly and white-core grains, this study was conducted to compare the physicochemical properties of white-belly, white-core, and their counterpart translucent grains, with the aim of examining the biochemical foundation of grain chalkiness.

## 2. Material and methods

### 2.1. Material

Two mutants (WBRK and WCRK) with respect to grain chalkiness from a japonica rice cultivar Wuyujing3, as reported by Zhang et al. (2014), were used in the study. WBRK has high ratio of white-belly grains, while WCRK has high ratio of white-core grains. Moreover, unlike conventional cultivars with a mixed type of grain chalkiness, WBRK only has one type of grain chalkiness, the white-belly. WCRK has both white-core and white-belly, but the ratio of white-belly was much lower. In 2013, WBRK had more than 61% white-belly grains and none white-core grains. In contrast, WCRK had approximately 57% white-core grains, and only 3% white-belly grains (Supplementary Table S1). The appearances of the dehulled grains were clearly showed in Supplementary Fig. S1. Therefore, these two mutants are reasonable for study on white-belly and white-core.

The two mutants were planted in the pots with 20 cm diameter at Danyang Experimental Station (31°54′31″N, 119°28′21″E) of Nanjing Agriculture University in 2013. Each pot was filled with 15 kg soil and 5 seedlings were planted in the form of each seedling per hill. Rice seeds were sown on April 23, and the seedlings were transplanted to the pots on May 27. In each pot, 1.5 g and 1.0 g nitrogen were applied as basal fertilizer and panicle fertilizer, respectively. During the flowering, we marked the grains on top three primary rachis that were found with high incidences of chalk, according to the finding of positional variation in white-belly and white-core rice kernel within a rice panicle (%). And then, the marked grains were collected at 4, 8, 12, 16, 20, 25, 30 and 35 days after flowering (DAF), dried, dehulled and weighted. At maturity, rice grains were randomly harvested, dried and stored at room temperature for 3 months, and then dehulled (JLG-II Test Husker, Zhejing). Grains of WBRK were separated into two subsamples, white-belly grains and translucent grains, while those of WCRK two subsamples, white-core grains and translucent grains. The embryo of the four subsamples was removed by a razor blade, and the remaining endosperm was ground to pass a 100-mesh sieve.

### 2.2. Grain weight and kernel size

A total of 1000 grains were randomly counted from each sample and weighed to determine grain weight. The grain length, width, and thickness of 20 dehulled whole grains from each sample were measured using vernier caliper. The ratio of grain length to width (L/B ratio) was calculated.

### 2.3. Starch and proteins

Total starch content was determined using an automatic polarimeter (WZZ-2B, Shanghai, China). Amylose content was analyzed according to Juliano's method (Juliano, 1971), and amylopectin content were calculated by subtraction of amylose content from total starch content.

Protein fractions were separated and analyzed followed by the method described by Liu et al. (2005). Albumins, globulins, prolamins and glutelins were extracted from rice power by water, 10% NaCl, 55% n-propanol and Biuret reagent in sequence. Glutelin content was determined by the Biuret method (Holme and Peck, 1998), and the others were determined using the Bradford reagent described by Bollag and Edelstein (1990). Total protein content was calculated as the sum of the four fractions with three replicates.

### 2.4. Amino acids

Amino acids analysis was performed using an L-8900 High-Speed Amino Acid Analyzer (Hitachi, Japan) after acid hydrolysis. Before analysis, 100 mg rice flour was hydrolyzed with 10 mL 6 mol/L HCl under vacuum at 110 °C for 24 h. Sample volumes were then adjusted to 100 mL with purified water. 1 mL solution was taken out for vacuum drying at 40 °C, and the residue was dissolved in 1 mL 0.2 mol/L HCl and loaded on amino acid analyzer. The amino acid contents were measured in duplicate. During acid hydrolysis, asparagine and glutamine undergo deamidation, resulting in aspartate and glutamate, respectively. Thus, the acids and their corresponding amides are indistinguishable. Tryptophan and cysteine are destroyed under acid hydrolysis conditions.

### 2.5. Minerals

For mineral analysis, inductively coupled plasma optical emission spectrometry (ICP-OES) was used to determine the contents of macronutrients (calcium, Ca; magnesium, Mg; potassium, K; sodium, Na) and micronutrients (copper, Cu; iron, Fe; manganese, Mn; zinc, Zn) in the dried rice flour. Each sample was measured in triplicate.

### 2.6. Statistical analysis

The data shown are an average of triplicate except amino acids that are means of duplicate measurements. The variance analysis was performed using Duncan's new multiple-range test in SPSS 16.0 statistical software.

## 3. Results

### 3.1. Changes in dry weight during caryopsis development

Changes in dry weight during caryopsis development varied with WBRK and WCRK (Supplementary Fig. S2). Dry weight increased rapidly as the growth and development of caryopsis progressed. Marked differences were found between WBRK and WCRK, with the caryopsis dry weight of WCRK being higher than WBRK before 16 DAF, and afterwards lower than WBRK.

### 3.2. Physical properties of white-belly and white-core grains

Table 1 shows the differences between white-belly, white-core and their counterpart translucent grains in physical properties. White-belly grains were larger in length, width and thickness, but lower in L/W ratio than the translucent ones. White-core grains were similar to white-belly grains, and were larger in kernel size and lower in L/W ratio than the translucent. In addition, grain weights of both chalky and translucent grains varied from 23.02 g to 25.93 g, with white-belly grains from WBRK having the highest